

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Satoko YAMAHIRA, et al.

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Examiner: Irene MARX

For: LACTIC ACID BACTERIA CAPABLE OF STIMULATING MUCOSAL
IMMUNITY

DECLARATION UNDER 37 C.F.R. § 1132

Honorable Commissioner of Patents and Trademarks

Washington, D.C. 20231

SIR:

I, Satoko YAMAHIRA declare that:

- 1) I am one of the inventors of the above-identified application, and am familiar with the subject matter of said application as well as the disclosures in the cited references.
- 2) In order to demonstrate the advantage of the present invention, the following experiments were carried out under my direction and supervision.

Experimental Report

Method

Sixty strains in total, including 57 *Lactobacillus plantarum* strains, were obtained, and, using IgA S.I., IgA production of each strain was compared. IgA concentration in culture supernatant was measured in accordance with the method described in the present specification, page 39, line 16 to page 40, line 7 of the English specification.

Results

A measured value of the culture supernatant obtained by adding 10 μ l of PBS(-) to an MEM medium (no bacteria added) and performing culture for seven days was defined as the standard (1.0), and the relative ratios (Stimulation Index:S.I.) were calculated to compare IgA S.I. in each strain (English specification, page 40, lines 9 to 19). The measurement results are shown in Table I below.

[Table I]

Table I: IgA production enhancing activity of 60 strains, using a Peyer's patch cell culture system

Strain No.	<u>Genus</u>	Species	IgA S.I.
	Control (PBS)		1.00
	Positive Control (LPS)		7.42
ONRIC b0239	<i>Lactobacillus</i>	<i>plantarum</i>	5.61
ONRIC b0240	<i>Lactobacillus</i>	<i>plantarum</i>	6.31
WON 0001	<i>Lactobacillus</i>	<i>plantarum</i>	2.46
WON 0010	<i>Lactobacillus</i>	<i>plantarum</i>	2.44
WON 0011	<i>Lactobacillus</i>	<i>plantarum</i>	1.24
WON 0030	<i>Lactobacillus</i>	<i>plantarum</i>	1.72
WON 0042	<i>Lactobacillus</i>	<i>plantarum</i>	1.17
WON 0052	<i>Lactobacillus</i>	<i>plantarum</i>	1.10
WON 0084	<i>Lactobacillus</i>	<i>plantarum</i>	1.61
WON 0102	<i>Lactobacillus</i>	<i>plantarum</i>	1.02
WON 0107	<i>Lactobacillus</i>	<i>plantarum</i>	0.94
WON 0111	<i>Lactobacillus</i>	<i>plantarum</i>	1.57
WON 0112	<i>Lactobacillus</i>	<i>plantarum</i>	1.38

WON 0120	<i>Lactobacillus</i>	<i>plantarum</i>	1.46
WON 0138	<i>Lactobacillus</i>	<i>plantarum</i>	1.72
WON 0139	<i>Lactobacillus</i>	<i>plantarum</i>	1.22
WON 0147	<i>Lactobacillus</i>	<i>plantarum</i>	0.97
WON 0158	<i>Lactobacillus</i>	<i>plantarum</i>	1.17
WON 0281	<i>Lactobacillus</i>	<i>plantarum</i>	1.82
WON 0288	<i>Lactobacillus</i>	<i>plantarum</i>	1.08
WON 0351	<i>Lactobacillus</i>	<i>plantarum</i>	2.09
WON 0352	<i>Lactobacillus</i>	<i>plantarum</i>	1.36
WON 0360	<i>Lactobacillus</i>	<i>plantarum</i>	1.40
WON 0364	<i>Lactobacillus</i>	<i>plantarum</i>	1.28
WON 0365	<i>Lactobacillus</i>	<i>plantarum</i>	0.78
WON 0366	<i>Lactobacillus</i>	<i>plantarum</i>	0.64
WON 0370	<i>Lactobacillus</i>	<i>plantarum</i>	1.07
WON 0373	<i>Lactobacillus</i>	<i>plantarum</i>	0.63
WON 0374	<i>Lactobacillus</i>	<i>plantarum</i>	1.28
WON 0387	<i>Lactobacillus</i>	<i>plantarum</i>	0.95
WON 0392	<i>Lactobacillus</i>	<i>plantarum</i>	0.80
WON 0401	<i>Lactobacillus</i>	<i>plantarum</i>	0.84
WON 0425	<i>Lactobacillus</i>	<i>plantarum</i>	1.15
WON 0432	<i>Lactobacillus</i>	<i>plantarum</i>	1.97
WON 0499	<i>Lactobacillus</i>	<i>plantarum</i>	0.96
WON 0505	<i>Lactobacillus</i>	<i>plantarum</i>	0.64
WON 0521	<i>Lactobacillus</i>	<i>plantarum</i>	1.19
WON 0533	<i>Lactobacillus</i>	<i>plantarum</i>	1.02
WON 0535	<i>Lactobacillus</i>	<i>plantarum</i>	0.75
WON 0536	<i>Lactobacillus</i>	<i>plantarum</i>	1.53
WON 0558	<i>Lactobacillus</i>	<i>plantarum</i>	0.89
WON 0568	<i>Lactobacillus</i>	<i>plantarum</i>	1.24
NRIC 1554	<i>Lactobacillus</i>	<i>plantarum</i>	1.00
NRIC 1758	<i>Lactobacillus</i>	<i>plantarum</i>	1.00
NRIC 1759	<i>Lactobacillus</i>	<i>plantarum</i>	1.27
NRIC 1925	<i>Lactobacillus</i>	<i>plantarum</i>	1.03
NRIC 1926	<i>Lactobacillus</i>	<i>plantarum</i>	1.02
AHU 1089	<i>Streptococcus</i> I	<i>lactis</i>	1.15

AHU 1257	<i>Streptococcus</i>	<i>faecalis</i>	1.04
AHU 1696	<i>Lactobacillus</i>	<i>casei</i>	1.01
ONRICb0315	<i>Lactobacillus</i>	<i>plantarum</i>	1.02
ONRICb0316	<i>Lactobacillus</i>	<i>plantarum</i>	0.89
ONRICb0317	<i>Lactobacillus</i>	<i>plantarum</i>	1.20
ONRICb0318	<i>Lactobacillus</i>	<i>plantarum</i>	1.57
ONRICb0319	<i>Lactobacillus</i>	<i>plantarum</i>	1.38
ONRICb0320	<i>Lactobacillus</i>	<i>plantarum</i>	1.44
ONRICb0321	<i>Lactobacillus</i>	<i>plantarum</i>	1.45
ONRICb0322	<i>Lactobacillus</i>	<i>plantarum</i>	1.47
ONRICb0323	<i>Lactobacillus</i>	<i>plantarum</i>	1.36
299v	<i>Lactobacillus</i>	<i>plantarum</i>	2.16

The abbreviations shown under "Strain No." in the table stand for the following microorganism depositories:

ONRIC: Otsu Nutraceuticals Research Institute Collection

WON: Wild strain of Otsu Nutraceuticals Research Institute Collection

AHU: Agriculture Hokkaido University

NRIC: NODAI Culture Collection Center, Tokyo University of Agriculture; Setagaya-ku, Tokyo, Japan

Table I shows that the only strains having an IgA S.I. value of 5 or more are *Lactobacillus plantarum* ONRIC b0239 and b0240, which are both lactic acid bacteria of the present invention.

As described above, the lactic acid bacteria strain of the present invention has especially excellent IgA production-enhancing activity among a number of existing *Lactobacillus plantarum* lactic acid bacteria strains. Even in comparison with known lactic acid bacteria, the IgA production-enhancing activity of the strain of the present invention is clearly higher.

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I, undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 18/2/2009

Satoko Yamahira

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